HB P-NILOTIC IN ASSOCIATION WITH β^0-THALASSEMIA: *CIS*-MUTATION OF A HEMOGLOBIN β^A CHAIN REGULATORY DETERMINANT?

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Reprinted from
THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE
St. Louis

Vol. 93, No. 6, pp. 973-982, June, 1979

Hb P-Nilotic in association with β^0-thalassemia: *cis*-mutation of a hemoglobin β^A chain regulatory determinant?

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Hb P-Nilotic which is produced by a hybrid of β and δ genes was found in several members of a Sudanese family, three of whom had an associated β-thalassemia. Chemical analyses confirmed the crossover between positions 22 and 50 of the $\beta\delta^P$ chain. The Hb P-Nilotic heterozygote had completely normal hematology, but the patients with the Hb P-Nilotic–β-thalassemia condition had moderately severe clinical and hematological abnormalities which were considerably more pronounced than those in the father who had a β-thalassemia heterozygosity. The absolute cellular contents of normal and abnormal non-α chains in these subjects and the results of in vitro chain synthesis analyses suggested that the thalassemia gene in this family is of the β^0 type and that the β^A gene which is present *in cis* to the $\beta\delta^P$ gene is incapable of being stimulated when the β^0-thalassemia determinant is present *in trans*. It is proposed that a number of recombination events produced a $\beta\delta^P$ hybrid gene with duplication of the β^A gene *in cis* as well as a change in an untranscribed strand of DNA which controls the expression of the β^A gene. (J LAB CLIN MED 93:973, 1979.)

Abbreviations: hemoglobin, with notation only (Hb), acid-citrate-dextrose (ACD), diethylaminoethyl (DEAE), half-life ($t_{1/2}$), carboxymethyl (CM), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH)

The genes directing the synthesis of the β and δ chains of human hemoglobin are closely linked on chromosome No. 11.[1] Abnormal recombination between these two structural genes caused by a mismatching due to base-sequence similarities can result in the formation of hybrid genes. One of these, the $\delta\beta$ fusion gene, produces a $\delta\beta$ chain found in the Lepore hemoglobins.[2] Because homozygotes for this type of hybrid hemoglobin have Hb Lepore and Hb F but no Hb A and Hb A_2, complete β and δ structural genes must certainly be absent.[3] Other crossover products are the hemoglobins Miyada[4] and P-Nilotic[5–7]; these "anti-Lepore" hemoglobins contain normal α chains and $\beta\delta$ chains.

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This research was in part supported by U.S. Public Health Service research grants HLB-05168 and HLB-15158.
Submitted for publication Sept. 18, 1978; accepted Feb. 28, 1979.
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Table I. Hematological and hemoglobin composition data on several members of the family with Hb P-Nilotic and β-thalassemia

Case[A]	Condition	*Hb* (*gm/dl*)	*PCV* (*1/1*)	*RBC* (10^{12}/*1*)	*MCV* (*fl*)	*MCH* (*pg*)
II-3	A–β-thal	9.5	0.282	4.34	65	22.0
II-5	AA[C]	9.6	0.295	3.20	92	30.3
III-1	P–β-thal	8.9	0.274	4.35	63	20.5
III-2	AP	12.8	0.381	4.36	85	27.7
Second sample[D]		12.6	0.376	4.14	91	30.6
III-3	A–β-thal	11.1	0.345	5.81	59	18.2
III-5	AA[C]	10.6	0.322	3.48	92	30.7
III-6	AA[C]	13.1	0.392	4.28	92	30.8
III-7	AA[C]	9.9	0.323	4.02	80	24.7
IV-1	P–β-thal	9.6	0.300	4.66	63	19.5
Second sample[D]		10.3	0.327	4.82	68	21.4
IV-2	P–β-thal	7.5	0.231	3.92	58	18.0
Second sample[D]		9.2	0.284	4.78	60	19.3
IV-3	AA[C]	14.6	0.437	5.08	86	29.1
IV-7	AP	10.0	0.264	3.16	84	31.9

PCV = packed cell volume; RBC = red blood cell; MCHC = mean corpuscular hemoglobin concentration.
[A]See pedigree in Fig. 1; ages are known for II-3 (90 years), III-2 (50 years), III-3 (56 years), IV-1 (26 years), and IV-2 (18 years).
[B]By DEAE-cellulose chromatography; %A includes minor A_1 components.
[C]These persons without a hemoglobinopathy are included to serve as internal controls; three of them, all female over 40 years of age, had a slight anemia probably of nutritional origin.
[D]Collected after a 3-month interval.

Dherte et al.[5] described one person who had Hb A, Hb P-Nilotic, and Hb S, and thus three active structural non-α genes. This is considered strong evidence in support of the hypothesis that a recombination event during a crossover involving the δ and β structural genes can produce a chromosome on which an "anti-Lepore" (βδ) gene is present together with intact δ and β structural genes, i.e., a duplication chromosome.[6]

Lepore hemoglobins are known to occur in association with β-thalassemia.[3] The syndrome, which has been described in Greeks, Italians, and Yugoslavs (for a review see refs. 3 and 8) and in New Guineans[9] gives rise to a clinical condition that resembles homozygous β-thalassemia. We have studied several members of a large family of Northern Sudan who were heterozygous for Hb P-Nilotic alone or in association with a β^0-thalassemia. Unexpectedly, the patients with the Hb P-Nilotic–β^0-thalassemia condition had a moderately severe thalassemia syndrome. Data from structural, hematological, and biosynthetic studies were consistent with the hypothesis that multiple recombination events resulted in the formation of the $\beta\delta^P$ hybrid gene with an additional β^A gene *in cis* to this gene, and of a modification of an untranscribed strand of DNA which has regulator function and which lies 5′ to the β^A gene. Unlike other β genes, the β^A gene *in cis* to the $\beta\delta^P$ gene is repressed and almost not inducible when a β^0-thalassemia determinant is present *in trans*.

Materials and methods

Blood samples. These were collected in ACD, mailed by air on ice, special delivery, to Augusta, Georgia, and arrived within 4 days. Informed consent was obtained from the donors according to the principles of the declaration of Helsinki prior to venepuncture. Occasionally the shipment included ^{14}C-labeled, saline-washed red cells. Hematological indices were obtained with a Coulter model S cell

MCHC (%)	*Hb* $A_2^{(B)}$ (%)	*Hb* $P^{(B)}$ (%)	*Hb* $A^{(B)}$ (%)	*Hb* $F^{(B)}$ (%)	*MCH-*$\beta\delta^P$ *(pg/cell)*	*MCH-*β^A *(pg/cell)*
33.4	5.6	—	—	<1.0	—	10.3
32.3	2.6	—	—	<1.0	—	—
32.1	5.75	55.65	31.9	6.7	5.7	3.27
32.8	3.0	27.9	69.1	<1.0	3.86	9.57
33.2	3.25	33.25	63.5	<1.0	5.09	9.72
37.2	5.8	—	—	<1.0	—	8.5
32.7	2.95	—	—	<1.0	—	—
33.2	3.15	—	—	<1.0	—	—
30.3	2.65	—	—	<1.0	—	—
31.3	6.2	57.5	32.7	3.4	5.61	3.19
31.3	6.2	57.6	33.1	3.1	6.16	3.54
31.5	6.1	51.1	34.4	8.4	4.60	3.10
31.9	5.95	51.7	34.2	8.2	4.99	3.30
33.3	2.6	—	—	<1.0	—	—
37.4	2.7	25.0	72.34	<1.0	3.98	11.5

counter (Coulter Electronics, Inc., Hialeah, Fla.). The clinical studies were done in Khartoum, Sudan, by standard methods.[10]

Hemoglobin analyses. (For references to methodology see ref. 11.) Electrophoretic examination of the hemoglobin was made by starch-gel electrophoresis at pH 9.0 and by citrate agar electrophoresis at pH 6.1. The various hemoglobin components were quantitated by DEAE-cellulose chromatography with glycine-KCN-NaCl developers. The same method, but on a preparative scale, was used to isolate the variant in larger quantities from blood samples of the patient, her brother, her uncle, and her mother. Globin was prepared by the method of Anson and Mirsky, and the chains were separated by CM-cellulose chromatography. After aminoethylation with ethylenimine, about 200 mg of the non-α chain (referred to as $\beta\delta^P$ chain) was available for structural analyses. Part of this material was digested with trypsin, and the tryptic peptides were separated on a 0.9 by 60 cm column of Chromobead resin, type P, at 50° C with pyridine-acetic acid volatile buffers. Isolated peptides were hydrolyzed with 6M HCl under reduced pressure at 110° C for 24 hr and occasionally for 72 hr. Amino acid analyses were made with a Spinco Model 121M automated amino acid analyzer equipped with high-sensitivity cuvettes and an integrator. The first 40 residues of the chain were also put in sequence by means of a Beckman sequencer (Model 890C; Beckman Instruments, Inc., Palo Alto, Calif.) equipped with an improved program (102974-576). PTH amino acid residues were analyzed after back hydrolysis with HI by means of the Spinco Model 121M amino acid analyzer.

Similar structural analyses were made on the β^A chain of Hb A isolated from the blood of the propositus (IV-2) with the Hb P-Nilotic–β^0-thalassemia condition.

Biosynthetic analyses. Red cells from venous blood or bone marrow aspirate were washed three times with cold NKM solution (NaCl 8.18 gm/L; KCl 0.37 gm/L; $MgCl_2 \cdot 6\ H_2O$ 1.42 gm/L) and were incubated in Dr. Abu-Sin's laboratory at Khartoum, Sudan, using the amino acid mixture with ^{14}C-leucine as described elsewhere.[11] Aliquots were taken at different times as indicated later, washed with saline solution, and shipped by air to Augusta, Georgia. All red cell aliquots were lysed with equal volumes of water, and each of the resulting whole cell lysates was divided into two samples for preparation of stroma-free hemolysate and whole cell globin, respectively. Stroma-free hemolysates were made by centrifuging whole cell lysates at 20,000 × g and 4° C for 30 min in a Sorval RC2 centrifuge (Du Pont Instruments—Sorvall, Wilmington, Del.). Hemoglobins in these hemolysates were isolated by chromatography on columns of DEAE-cellulose.[11] Aliquots (1 ml) of effluent fractions of the columns were treated with perchloric acid and hydrogen peroxide for liquid scintillation counting. Whole cell globins were made by precipitation of whole cell lysates with acid acetone. Globin was

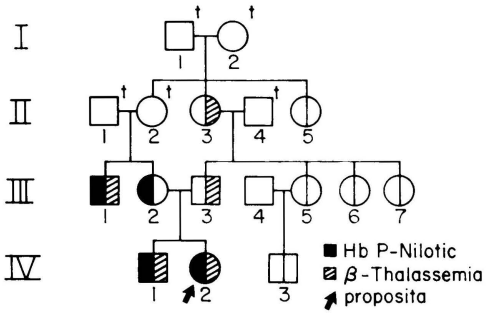

Fig. 1. Pedigree of the family with Hb P-Nilotic and β-thalassemia.

also made from isolated hemoglobin. These globins were analyzed by chromatography on CM-cellulose with β-mercaptoethanol-phosphate-urea buffers.[11]

Results

Case and family report. Part of the pedigree of this large family is shown in Fig. 1. Patient H. A. R. (Case IV-2), an 18-year-old girl from Northern Sudan, was referred to one of us (A. A. S.) in June 1976 because of refractory anemia and splenomegaly which were thought to be present since early childhood. Close questioning indicated no history of blood loss or febrile episodes, and her diet was described as adequate. She had never received a blood transfusion. Physical examination showed mild pallor and a spleen which was palpable 6 cm below the left costal margin.

Blood counts remained fairly constant over the period of May 1976 to June 1977. Representative hematological values, determined in June 1977, are given in Table I. The WBC count was 6.0×10^9/L with a normal differential; the platelet count was 148×10^9/L, and the reticulocyte count was 6%. On a peripheral blood film, marked anisopoikilocytosis, mild to moderate hypochromia, few nucleated red cells, and basophilic stippling were apparent. Bone marrow examination showed normoblastic erythroid hyperplasia. The serum iron level was 69 μmol/L (385 μg/100 ml). Red cell survival studies with ^{51}Cr as a label showed a t½ of 18 days (normal 28 to 30) with an increased uptake of the radioisotope by the spleen.

The patient's brother (M. A. R., Case IV-1), 26 years old, was not available for complete investigation. Although he denied symptoms of anemia, he had consulted a surgeon on occasion because of dull pain and heaviness in the left hypochondrium. Physical examination revealed an enlarged spleen 4 cm below the left costal margin. He was anemic (Table I) although somewhat less severely than his sister. The WBC count was 4.4×10^9/L, with a normal differential and a normal platelet count. His blood film showed a moderate to marked poikilocytosis with mild hypochromia. An uncle (Case III-1) had similar hematological and clinical features. The mother (Case III-2), 50 years old, enjoyed good health and was without physical abnormality. The father (Case III-3), 55 years old, had diabetes mellitus but was otherwise normal. Hematological values for the parents are included in Table I; the father and mother are first cousins (Fig. 1).

Hemoglobin structure. When the hemoglobins from several members of this family were examined by electrophoresis and chromatography, a variant with properties somewhat similar to those of Hb S was observed in Cases III-1, III-2, IV-1, and IV-2 (and three others). The tryptic peptides of the abnormal non-α chain of this variant were isolated by chromatography. Peptides T-1, T-4, T-6, T-7, T-8,9, T-11, T-12a, T-14, T-15, and T-14,15,

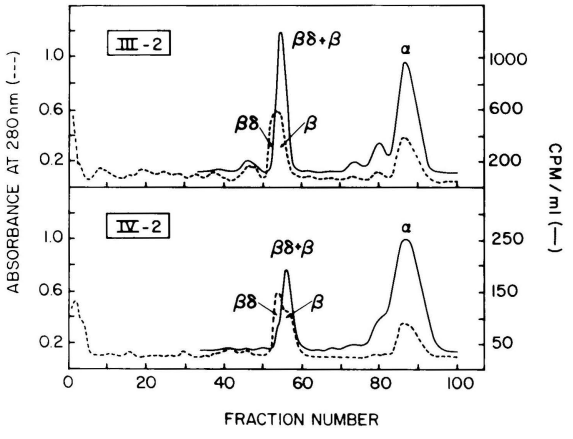

Fig. 2. Separation of globin chains on columns of CM-cellulose. Case III-2 had a Hb P-Nilotic trait with 28% Hb P, and Case IV-2 had the Hb P Nilotic–β-thalassemia condition with 55% Hb P. It was not possible to obtain separation of the $\beta\delta$ chain of Hb P-Nilotic and the β^A chain of Hb A by this technique, although the presence of the $\beta\delta^P$ chain could be appreciated when its level in the sample was high.

which are identical in the normal β chain and δ chain, had the correct composition (including the two valyl residues in T-4 and the three valyl residues in T-14 and T-14,15). The compositions of T-2 and T-3 coincided with those of the normal β chain, whereas the compositions of the T-5, T-10, T-10,11, T-12b, T-12c, and T-13 peptides agreed with the corresponding peptides of the δ chain. These data indicate that the sequence of residues 1 to 22 is the sequence of the β chain and that of residues 50 to 146 is the sequence of the δ chain. This sequence corresponds to the described structure of the $\beta\delta^P$ chain of Hb P-Nilotic.[6] The results of the automatic sequence determination of the first 40 residues corroborated these findings. All tryptic peptides of the β chain of the proposita's Hb A had the amino acid composition expected for corresponding fragments of the normal β^A chain (detailed results of these structural analyses will be provided upon request).

Hemoglobin analyses. Hemoglobin composition and hematological data are listed in Table I. The mother (Case III-2) appears to be a Hb P-Nilotic heterozygote, and the father (Case III-3) appears to be a β^0-thalassemia heterozygote (on the basis of the elevated level of Hb A_2, the extremely low MCV and MCH values, and the biosynthetic data to be presented later). The two children (Cases IV-1 and IV-2) and the uncle (Case III-1) have the Hb P-Nilotic–β^0-thalassemia condition. The level of Hb A_2 in these three patients was about 6% and that of Hb F was below 10%, the average values for Hb P-Nilotic and Hb A were 55% and 33%, respectively, and the MCV and MCH values were as low as seen in the father. The average amount of $\beta\delta^P$ chain per cell was 5.4 ± 0.6 (S.D.) pg (n = 3), and that of the β^A chain was 3.3 ± 0.15 pg (n = 3). The level of Hb P-Nilotic in the mother and three distant relatives with the same Hb P-Nilotic heterozygosity averaged 28.0% ± 1.9 (n = 4), and that of Hb A_2 was 2.85% ± 0.25 (n = 4). The average amount of $\beta\delta^P$ chain per cell was 4.3 ± 0.7 pg (n = 3), and that of the $\beta^A$was 10.3 ± 1.0 pg (n = 3).

Biosynthetic analyses. Examples of chromatographic analyses of whole cell globin are given in Fig. 2. Since the separation of the $\beta\delta^P$ and β^A chains was only partial and always inadequate for separate calculations of the radioactivity in the respective chromatographic zones, only the ratios of the α and the total non-α (β^A + $\beta\delta^P$) radioactivities are

Table II. Biosynthetic data on whole cell globin from peripheral blood of five members of the family with Hb P-Nilotic and β^0-thalassemia[A]

Subject	*Condition*	*Time of incubation (min)*	$\alpha^{(B)}$	$\beta + \beta\delta^{(B)}$	$\frac{\alpha}{\beta + \beta\delta^{(C)}}$
III-2	A-P	30	6,948	5,224	1.33
		60	10,456	6,440	1.62
		120	26,116	12,900	2.02
III-3	A–β-thal	30	7,108	3,316	2.14
		60	13,088	6,568	1.99
		120	12,760	7,184	1.78
IV-1	P–β-thal	30	20,736	4,436	4.67
		60	7,216	2,180	3.31
		120	53,628	13,636	3.84
IV-2	P–β-thal	30	8,252	2,600	3.17
		60	19,172	4,740	4.04
		120	29,432	7,420	3.97
III-1	P–β-thal	30	7,784	1,836	4.24
		60	30,948	6,812	4.54
		120	21,348	5,688	3.75

[A]The amount of globin used in each analysis varied from 20 to 40 mg.
[B]Data are in cpm in the respective zones. Since the β and $\beta\delta$ chains did not separate, the total non-α chain activity is listed (see also Fig. 2).
[C]Average value for Case III-3 is 1.97 ± 0.18; average values for Cases IV-1, IV-2, and III-1 are 4.0 ± 0.8 at 30 min, 4.0 ± 0.6 at 60 min, and 3.9 ± 0.1 at 120 min.

listed in Table II. The mother (Case III-2) had an increasingly imbalanced synthesis at longer times of incubation. The father (Case III-3) had an average α/non-α total activity ratio of about 2.0, although a minimal decrease in the ratio was noted during prolonged incubation. The patients with Hb P-Nilotic–β^0-thalassemia (Cases IV-1, IV-2, and III-1) had average ratios of 3.9, 3.7, and 4.2, respectively.

The distribution of radioactivity in the Hb A and Hb P-Nilotic in peripheral blood is presented in Table III. The ratio of the total radioactivities incorporated into the Hb A and Hb P-Nilotic zones was 10 for Case III-2 with the Hb P-Nilotic trait but was only 2.1 and 3.2 for Cases IV-2 and III-1 with the Hb P-Nilotic–β^0-thalassemia condition. A bone marrow sample obtained from Case III-1 and incubated for only 15 min gave a Hb A/Hb P-Nilotic ratio of only 1.66. However, further analyses of the isolated Hb A fraction by CM-cellulose chromatography showed the presence of as much as 25% radioactivity due to an unidentified nonhemoglobin protein. Such a component was not identified in the Hb P-Nilotic fraction. The corrected value for the Hb A/Hb P-Nilotic ratio in bone marrow is therefore about 1.2. Analyses of the isolated hemoglobins from this bone marrow sample gave an α/non-α value of 0.2 for Hb P-Nilotic and 1.5 for Hb A. In peripheral blood, the values were 2.1 to 2.9 for Hb P-Nilotic and 0.8 to 1.0 for Hb A. This indicates that the production of $\beta\delta^P$ chains was more effective in bone marrow than in peripheral blood. In fact, the specific activities of the $\beta\delta^P$ chain and the β^A chains which were separated from Hb P and Hb A having been isolated from peripheral blood samples gave a $\beta\delta^P/\beta^A$ ratio of 0.15 for one of the Hb P heterozygotes and an average ratio of 0.22 for two cases with Hb P–β^0-thalassemia. The corresponding value in the bone marrow aspirate of the Hb P–β^0-thalassemia patient was 0.49. These data make it most unlikely that the relative proportions of Hb P and Hb A in the two types of conditions were altered after translation as is the case for various unstable hemoglobins (for reference, see ref. 12).

Table III. Biosynthetic data on the hemoglobin components isolated from blood and bone marrow samples of three members of the family with Hb P-Nilotic trait or with Hb P-Nilotic–β^0-thalassemia[A]

Case	Condition	Material	Time of incubation (min)	Total activity[B]		
				Hb P[C]	Hb A[C]	A/P[D]
III-2	A-P	Blood	30	1,300	13,100	10.1
			60	1,220	11,610	9.5
			120	2,480	25,560	10.3
IV-2	P–β-thal	Blood	30	10,930	23,700	2.2
			60	16,040	34,410	2.0
			120	9,610	20.830	2.2
III-1	P–β-thal	Blood	60	1,580	5,360	3.4
			120	4,320	13,510	3.1
		Bone marrow	15	3,600	4,440[E]	1.2

[A]The amount of hemoglobin chromatographed varied from 25 to 40 mg.
[B]Total cpm in the respective zones.
[C]Separated by DEAE-cellulose chromatography.
[D]Average value for Case III-2 is 10.0 ± 0.4 and 2.6 ± 0.6 for Cases IV-2 and III-1 (peripheral blood only).
[E]Corrected for presence of contaminant protein (see text).

Discussion

The hemoglobin variant. The abnormal hemoglobin in the proposita and other members of this family was identified as Hb P-Nilotic,[5] an anti-Lepore hemoglobin which, like Hb Miyada[4] and others, has a β chain sequence near the amino-terminus and a δ chain sequence near the carboxy-terminus. These abnormal hemoglobins are produced by hybrid genes which probably arise by abnormal recombination between homologous β and δ genes due to base mispairing during meiosis.[6] A similar mechanism may also result in the Lepore type of abnormal hemoglobins which have a δ chain sequence near the amino-terminus and a β chain sequence near the carboxy-terminus. However, the anti-Lepore type of abnormality cannot be considered as a "reverse Lepore" because a functioning β^A gene is present *in cis* to the $\beta\delta$ hybrid gene.[5] The mutation which is characteristic for Hb P-Nilotic can also be found in Hb Lincoln Park which, in addition, has a deletion of a valyl residue in position 137 of the $\beta\delta^P$ chain.[13] Structural analyses have shown that this second abnormality is absent in the $\beta\delta$ chain of the Hb P present in our family. Hb Lincoln Park is a stable hemoglobin variant with normal functional properties, has been found in several members of a Mexican family, and comprises about 14% of total circulating hemoglobin in heterozygotes.[13] The proportion of Hb P-Nilotic in the heterozygotes of the Sudanese family described here averaged 28% (Table I).

The mother with Hb P-Nilotic trait. Hb Lepore heterozygotes phenotypically resemble $\delta\beta$-thalassemia heterozygotes.[3] However, anti-Lepore heterozygotes such as the mother (Case III-2) of the present family did not have any hematological abnormality. The absence of a hemolytic syndrome in this person is consistent with normal erythropoiesis, and a balanced synthesis of hemoglobin chains would be expected. Nonetheless, progressive imbalance of chain synthesis was observed as the in vitro incubation of reticulocytes proceeded. Perhaps this may be explained by an early decline of the in vitro synthesis of $\beta\delta^P$ chains. Comparison of the in vitro synthesis data for bone marrow and peripheral blood suggests that the $\beta\delta$ chains of Hb P-Nilotic are synthesized to a much larger extent in nucleated erythroid precursors than in peripheral blood reticulocytes (see also Roberts et al.,[14] who studied the $\beta\delta$ chain of Hb Miyada).

The father with β^0-thalassemia trait. Hematological observations and data from in vitro synthesis experiments indicate that the father (Case III-3) has a β^0-thalassemia trait; the α/non-α ratio (total radioactivity) of 1.97 ± 0.18 (S.D.) (n = 3) is similar to that observed for a group of patients with a β^0 or $\delta\beta^0$-thalassemia trait with complete absence of β^A chain production *in cis* (1.96 ± 0.30; n = 9) and rather different from the ratio in β^+-thalassemia heterozygotes (1.23 ±0.05; n = 6). These control data were determined in heterozygotes of families in which Hb S and β-thalassemia genes were segregating or in parents of homozygous thalassemia patients without any β^A chain production (unpublished observation).

The members with Hb P-Nilotic–β^0-thalassemia. The separate heterozygosities in the mother (Case III-2) and the father (Case III-3) define the condition in the patients IV-1 and IV-2 (and also in III-1) as Hb P-Nilotic–β^0-thalassemia. The most interesting feature of this condition is the low content of Hb A. The small amounts of β^A chains (MCH-β^A = 3.3 ± 0.2 (S.D.) pg/cell) are produced only by the β gene *in cis* to the $\beta\delta^P$ hybrid gene. Data from chemical analyses leave little doubt that these are normal β^A chains. The Hb A/Hb P-Nilotic total activity ratio (2.6 ± 0.6; Table III) is about one fourth of the value observed in the Hb P-Nilotic heterozygote (10 ± 0.4). This difference is in close agreement with that which is observed between the ratios of the absolute amounts of β^A and $\beta\delta^P$ chains in peripheral red blood cells (Hb P–β^0-thalassemia cases: 0.6 ± 0.05; Hb P heterozygotes: 2.4 ± 0.5). Moreover, the in vitro synthesis imbalance is more severe than in either parent.

Genetic considerations. Mutant or normal β genes *in trans* to a β^0-thalassemia determinant usually compensate in part for the deficit of β chain synthesis. For instance, patients with the Hb S–β^0-thalassemia condition have a mean corpuscular β^S chain content (MCH-β^S) of about 9 pg/cell which is 50% higher than the 6 pg/cell of the Hb S heterozygote (ref. 12 and unpublished data). The two members of this family with heterozygous β^0-thalassemia averaged 9.5 pg of β^A chain per cell, which is similar to the values of MCH-β^S in patients with Hb S–β^0-thalassemia and less than the average 11 pg of β chains (β^A + β^S) per cell in patients with Hb S–β^+-thalassemia. Thus a normal or variant β gene *in trans* to a β^0-thalassemia determinant will produce as much as 9 pg of β chains per cell; however, the β^A gene which is *in cis* to the $\beta\delta^P$ gene and *in trans* to the β^0-thalassemia determinant in the three patients with the Hb P-Nilotic–β^0-thalassemia condition produces only one third of this amount. It should also be noted that the content of $\beta\delta^P$ chains in these patients (5.4 ± 0.6 (S.D.) pg; n = 5) is only slightly higher than that in the Hb P-Nilotic heterozygotes (4.3 ± 0.7 pg; n = 3); this difference is not significant (t = 1.87, d.f. = 4). Presumably, the $\beta\delta^P$ gene is maximally active or nearly so whether a normal or a β^0-thalassemia chromosome is *in trans* to it because of the deficiency of the β^A gene *in cis* to the $\beta\delta^P$ hybrid gene.

These considerations offer an explanation for the dependence of the clinical and hematological expression of the β^0-thalassemia determinant in this family on the type of chromosome that is present *in trans* to the thalassemia. Only a mild anemia exists in the β^0-thalassemia heterozygote with a normal δ locus and a normal β locus *in trans*, but when the δ-$\beta\delta^P$-β^Acomplex is present *in trans* (P-Nilotic–β^0-thalassemia), the clinical manifestations are more severe, and the in vitro synthesis imbalance is markedly increased at all incubation times. Indeed, the normal β^A gene *in cis* to the $\beta\delta^P$ gene must be repressed. Unlike other β genes this β^A gene is not derepressed to any great extent in a condition where a β^0-thalassemia is present *in trans*.

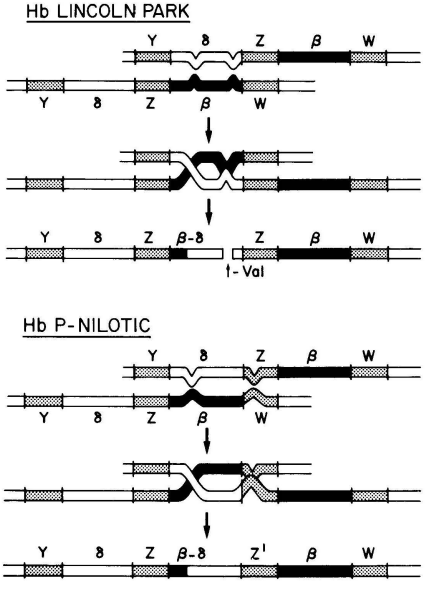

Fig. 3. Repeated crossing-over during meiosis as a possible mechanism for the development of the non-α chain in Hb Lincoln Park and Hb P-Nilotic. The sections *Y*, *Z*, and *W* are considered to be untranscribed strands of DNA between structural genes which have regulatory functions. For further details see text. (Modified from Honig GR, Shamsuddin M, Mason G, and Vida LN: Proc Sec Natl Acad Sci USA **75:**1475, 1978.)

The mechanism proposed to account for these observations is similar to that described to explain the structural characteristics of the $\beta\delta$ chain of Hb Lincoln Park.[13] The top section of Fig. 3 illustrates the occurrence of an unequal crossover between the β and δ genes resulting in the formation of a $\beta\delta$ hybrid gene and of two additional crossovers causing the deletion of the codon for valyl residue in position 137. Fig. 3 is a modification of Fig. 4 of ref. 13; the modification concerns the intergenic strands of DNA, designated *Y*, *Z* and *W*, which may have regulatory functions.[15] A single crossover between β and δ genes, as suggested by Badr et al.,[6] is clearly inadequate to explain our findings in the three patients with Hb P Nilotic–β^0-thalassemia because the duplicated Z strand would induce β^A gene activity *in cis* to the $\beta\delta^P$ gene which is contrary to what has been observed. More than one additional crossover may occur,[16] as is suggested in the lower part of Fig. 3. The additional postulated crossovers or other, ill-defined events occurred between untranscribed DNA sequences, Z and *W* in Fig. 3, resulting in a modified Z′ segment. One chromosome resulting from these recombinations carries a δ chain gene and a duplex of non-α genes, one of which is a $\beta\delta^P$ hybrid gene and the second a β^A gene that is now under repression by a mutated operator strand of DNA 5′ to it (segment Z′). Data from investigations on the structures of the mRNAs and restriction mapping of DNA will be the subject of a future report.

We are indebted to Dr. W. A. Schroeder for helpful discussion and to Dr. George Honig for a preprint of his article describing the discovery of Hb Lincoln Park. A. A. S. thanks Dr. W. Page Faulk of the Department of Immunology, Medical University of South Carolina, Charleston, S. C., for advice and encouragement.

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